



Identification and Age-Dependence of Pteridines in the Head of Adult Mexican Fruit Fly, *Anastrepha ludens*

N. TOMIC-CARRUTHERS,*†‡ D. C. ROBACKER,† R. L. MANGAN†

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Pteridines from head capsules of adult *Anastrepha ludens* were identified and evaluated as a tool for age determination of flies. Pteridines were identified by a combination of TLC, HPLC and UV and fluorescence spectroscopy. Tentative identifications were obtained for one red pterine, neodrosopterin, and three orange pterines, drosopterin, isodrosopterin and aurodrosopterin. Five pterines with blue or blue-green fluorescence were found and four were identified: 7,8-dihydrobiopterin; biopterin; pterin; and pterin-6-carboxylic acid. An additional blue fluorescing non-pteridine compound was identified as kyurenine. Two pterines with yellow fluorescence were identified as sepiapterin and deoxysepiapterin. Titers of sepiapterin and deoxysepiapterin were age dependent based on TLC and HPLC analyses. HPLC demonstrated that only deoxysepiapterin accumulated sufficiently and over a sufficiently long period of adult life to be useful as a quantitative tool for determining age of adult flies. The HPLC method developed in this work specifically for separation of deoxysepiapterin from sepiapterin and other pteridines used reverse-phase (C18) with a mobile phase of 30% methanol in water and detection at 420 nm.

Pteridines Sepiapterin Deoxysepiapterin Age *Anastrepha ludens*

INTRODUCTION

Fluorescent compounds from insects have been the object of investigations for almost a century. They were first studied as the chemical means of insect ornamentation (Hopkins, 1895), then as phenotypical expressions of polymorphism. Later, with the development of finer biochemical techniques, their physiology and metabolism were investigated. Most of the fluorescent compounds isolated from insects have been identified as pteridines, a name derived from the Greek "pteron" (wing), because of their natural occurrence in butterfly wings (Nixon, 1985). Structurally, they are based upon a pyrazino (2,3-d) pyrimidine ring system and are amphoteric compounds that are poorly soluble in water and most organic solvents.

Pteridines have been found in almost all insect groups and in various locations in the insects' bodies, although

the main locations of accumulation, at least in adult insects, are the head and the integument. The head capsules of some Diptera contain several different pteridines, most of which are C-6 substituted pterines in oxidized or hydrogenated forms. They are most concentrated in the eyes in the primary and secondary accessory cells of the ommatidium (Hearl and Jacobson, 1984). Physiologically, they remain only partially understood. They have been implicated as ornamental pigments in butterfly wings, as eye pigments in numerous insects and as cofactors in enzymatic processes associated with the metabolism of aromatic amino acids (Ziegler and Harmsen, 1969). They may also play a role in a specialized system of storage excretion (Harmsen, 1966).

Among applied entomologists, these fluorescent pteridines increased in importance after it was determined that the amount of fluorescence in extracts from the head of *Stomoxys calcitrans* increased directly with age (Mail *et al.*, 1983; Lehane *et al.*, 1986). Thus, these compounds provided a method for determining the age of some adult insects. This method has been successfully applied to several Diptera species: *Glossina morsitans morsitans* (Lehane and Mail, 1985); *Simulium damnosum* (Cheke *et al.*, 1990); *Glossina spp.* (Langley *et al.*, 1988);

*Texas A & M University Agricultural Research and Extension Center, 2415 E. Hwy 83, Weslaco, TX 78596, U.S.A.

†USDA-Agricultural Research Service, Crop Quality and Fruit Insects Research Unit Subtropical Agricultural Research Laboratory, 2301 S. International Blvd., Weslaco, TX 78596, U.S.A.

‡To whom all correspondence should be addressed.

Cochliomya hominivorax (Thomas and Chen, 1989); *Chrysomya bezziana* (Wall *et al.*, 1990); *Lucilia sericata* (Wall *et al.*, 1991); *Haematobia irritans irritans* (Krafsur *et al.*, 1992); and *Ceratitis capitata* (Camin *et al.*, 1991). Based on these studies, we hoped that it would be possible to use fluorescence measurements for age determination in the Mexican fruit fly (*Anastrepha ludens*) and other Tephritidae.

The technique developed by Lehane and Mail (1985) for age determination involved measuring fluorescence of all compounds extracted from the head, which, when excited with 360 nm light, emit at 450 nm. When used with *A. ludens*, this technique did not give satisfactory results. Although our experiments revealed that the intensity of fluorescence was increasing with time and physiological age, fluorescence readings were unstable and the change in fluorescence with age was not sufficient to be used for accurate age estimation. We hypothesized that the intensity of the fluorescence that we measured in *A. ludens* represented the contribution of several different pteridines that were present in either oxidized or reduced form. We further hypothesized that in the case of the Mexican fruit fly, pteridines that accumulate the most with age may be of a low fluorescing type (Ziegler and Harmsen, 1969) and thus were masked by other strongly fluorescing pteridines that are physiologically active but may not accumulate with adult age.

We know of no previous research on the pterine content in the head capsule of the Mexican fruit fly. This work on separation and identification of fluorescent compounds was conducted to test our hypothesis that some fluorescent compounds critical to age determination may be masked by other fluorescent compounds that do not increase in concentration through time. In addition, we believed that separation of specific pteridines and estimation of their accumulation with age would improve the standard method for age determination (Lehane and Mail, 1985) and make it suitable for use with the Mexican fruit fly and related species. At the same time, we felt that this work would contribute to the field of pteridine biochemistry and physiology and also would provide an introduction to our parallel research concerning the effects of age and different ecological factors on accumulation of specific pteridines.

METHODS AND MATERIALS

Flies

Mexican fruit fly (*A. ludens*) adults were obtained from the USDA, ARS Subtropical Agricultural Research Laboratory quarantine colonies in Weslaco, TX, where they were cultured using the techniques of Rhode and Spishakoff (1965). The adult flies were maintained at 28°C, relative humidity at about 80%, and a light regime of 12L : 12D under fluorescent lights. For studies to determine accumulation of pterines in the head capsule with age, groups of 200 flies were kept in 20 × 20 × 20 cm aluminum-screened cages. For identification of pterid-

ines, variable numbers of flies were kept in the cages. All adult flies were fed a diet containing yeast hydrolysate and sugar and were allowed continuous access to water.

Chemicals

Pterin, pterin-6-carboxylic acid, xanthopterin, isoxanthopterin and kynurenine were obtained from Sigma Chemical Co. (St Louis, MO, U.S.A.), biopterin from Aldrich Chemical Company (Milwaukee, WI, U.S.A.) and sepiapterin, deoxysepiapterin and 7,8-dihydro-L-biopterin from the laboratory of Dr B. Schircks (Jona, Switzerland). All chemicals used in extractions and the liquid phases of both chromatography systems were acquired from Aldrich or Sigma.

Extractions

Two types of extractions (A and B) were used for preparations of samples for thin-layer chromatography (TLC). Fifteen to twenty day old flies were decapitated and the head capsules were extracted immediately. Four fly heads were homogenized in a glass 200 µl tissue homogenizer either in: (A) 100 µl of propanol : 3.5% ammonium hydroxide (2 : 1) containing an additional 0.5 ml of 2-mercaptoethanol as a preservative (Wilson and Jacobson, 1977); or in (B) methanol : glacial acetic acid : water (4 : 1 : 5) (Ferre *et al.*, 1979). It was important to use both the alkaline (A) and acid (B) extractions because some pteridines are pH sensitive and decompose readily at one pH or the other (Merlini and Nasini, 1966). Homogenate was in both cases centrifuged in a microcentrifuge (5414 Eppendorf; Brinkman) at 12,000 g for 5 min to obtain a supernatant that was applied to TLC plates. The best results were obtained when samples were prepared on ice, in near darkness or indirect dim light, and analysed immediately. These two types of extractions were not used for HPLC because the resulting supernatants were not compatible with the reverse-phase (C-18) chromatography that was conducted.

For qualitative HPLC, (i.e. to identify pteridines associated with *A. ludens*), 15–20 day old flies were decapitated and the head capsules were extracted immediately as was done with TLC samples. Flies used in quantitative HPLC, (i.e. to determine accumulation of pteridines in the head capsule with aging), were removed from their holding cage every 3–4 days over a 30 day period for extraction. Five male and five female flies were collected during each sample period, freeze-killed, placed on silica gel in a sealed vial, and then stored at 4°C until chemical extractions and analyses were conducted. Decapitation was done immediately prior to extractions. As a control experiment for the effect of head size on amounts of pteridines, heads were dried and weighed before extraction in one series of fly-age samples.

All extracts for HPLC were prepared by a procedure modified from that of Lehane and Mail (1985) which was originally used for spectrofluorimetric analysis of head

extracts. Individual fly heads were homogenized in 50 μ l of chloroform : methanol (2 : 1) solution followed by addition of 75 μ l of 0.1 N NaOH adjusted to pH 10 with glycine. The resulting emulsion was shaken vigorously and centrifuged for 5 min at 12,000 g as described above. Supernatant was filtered through 0.45 μ cellulose acetate micro centrifuge filters and samples were placed on ice prior to injection.

Thin layer chromatography

Tentative identifications of the specific pteridines extracted from the fly heads were conducted by TLC. Precoated type 100 cellulose TLC plates (20 \times 10 cm; 0.25 mm layer thickness) (Sigma) were used for one dimensional chromatography, and precoated cellulose TLC plates (20 \times 20 cm; 0.5 mm layer thickness) (Merck) were used for two dimensional chromatography. The solvent system for one dimensional TLC was propanol : 1% ammonium hydroxide (2 : 1) (Collins and Kalnins, 1970). Two solvent systems used for two dimensional TLC were: (1) propanol : 1% ammonium hydroxide (2 : 1) in the first dimension and *n*-butanol : acetic acid : water (20 : 3 : 7) in the second dimension (Collins and Kalnins, 1970); and (2) isopropanol : ammonium acetate (1 : 1) in the first dimension and 3% ammonium chloride in the second dimension (Ferre *et al.*, 1986). Determination of the individual pteridines was conducted on the basis of side by side comparisons to synthetic standards (when available) or to extracts from the head capsules from *Drosophila melanogaster* on the basis of Rate factor (Rf) values and color of fluorescence under UV light.

High pressure liquid chromatography

HPLC was used primarily for determination of the effect of age on amounts of specific pteridines in fly heads. In certain cases for which synthetic standards were available, HPLC was also used in qualitative analysis for validation of the TLC results. For all studies, a reverse-phase C18 column (5 μ m particle size, 25 cm \times 4.6 mm) (Alltech Associates, Deerfield, Illinois, U.S.A.) was used with a Waters (Waters Milford, Massachusetts, U.S.A.) HPLC. Pteridines from the head extracts were separated in two different mobile phases and detected on two separate wavelengths. Analysis of pteridines in the bipterin-pterin group (the pteridines that fluoresce blue under UV light) was performed using 0.1% acetic acid : methanol (99 : 1 V/V) as mobile phase (Klein *et al.*, 1991) and detection at 360 nm (Multiwavelength M490 UV-VIS detector, Waters). Excellent separation of sepiapterin and deoxysepiapterin (the yellow fluorescing pteridines) was achieved using 30% methanol in water as the mobile phase and detection at 420 nm (M490). Results of the analyses including quantitations of sepiapterin and deoxysepiapterin were obtained with Millennium 2010 chromatography manager software (Waters). Identifications of pteridines were verified by comparing absorption spectra of separated compounds from head extracts

to synthetic standards using a Waters 996 Photodiode array detector.

RESULTS

Identification of pteridines

Between 10 and 12 fluorescent spots were separated on the cellulose plates using the two dimensional TLC solvent systems. All spots on the TLC plates that were clearly visible under UV light had either orange-red, blue or yellow fluorescence.

Red and orange pteridines were found in relatively small quantities. Rf values of these compounds were very low (Fig. 1.) in solvent system 1 (propanol : 1% ammonium hydroxide followed by *n*-butanol : acetic acid : water). The best resolution of orange and red pteridines was achieved with solvent system 2 (isopropanol : ammonium acetate followed by 3% ammonium chloride). The nonavailability of synthetic standards made positive identifications of these compounds less than conclusive. However, the color, distribution of spots on the plates and the color of their fluorescence suggest their identification as drosospterins. These compounds were compared to TLC separations of *Drosophila melanogaster* (Ferre *et al.*, 1986). Our separations revealed one red and three orange spots (indicated as 1-4, Fig. 1) and were tentatively determined as neodrosospterin (red spot) and as drosospterin, isodrosospterin and aurodrosospterin (three orange spots, respectively).

Excellent separation of yellow and blue fluorescent compounds was achieved in the two dimensional solvent system 1 (Fig. 1). Five compounds with blue fluorescence were distinguishable under UV light. The most fluorescent blue spot (no. 5 in Fig. 1) had Rf values corresponding in the first dimension with 7,8-dihydrobipterin and in the second dimension to neopterin. It was tentatively identified as neopterin that formed by spontaneous oxidation of 7,8-dihydrobipterin during chrom-

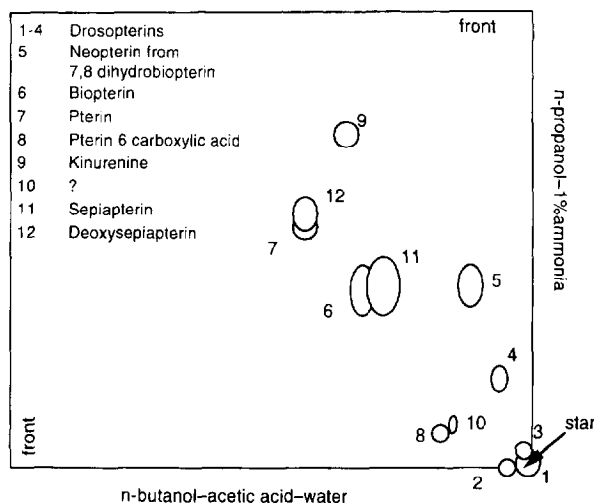


FIGURE 1. Thin layer chromatogram of the head extract from *A. ludens* seen under 365 nm UV light. Rf values of the spots on the chromatogram are in proportion to original plate.

atography, i.e. when plates were exposed to air between the first and second dimension runs. This oxidative process was demonstrated by Ferre *et al.* (1986). HPLC analysis using 0.1% acetic acid : methanol (99 : 1) as the mobile phase established that 7,8-dihydrobiopterin was present in unoxidized samples. This was demonstrated by comparison of retention times and absorption spectra of peak 1 from fly-head extracts [Fig. 2(A)] with retention times and absorption spectra of synthetic 7,8-dihydrobiopterin peak 1 in Fig. 2(B). Conversely, no neopterin was found in head extracts by HPLC analysis.

The blue spot labeled no. 6 (Fig. 1) had *R_f* values corresponding to biopterin in both dimensions. We hypothesize that some biopterin also formed by oxidation of 7,8-dihydrobiopterin and that biopterin partly accounts for spot no. 6. Oxidation of 7,8-dihydrobiopterin to biopterin also was demonstrated by Ferre *et al.* (1986). Nevertheless, HPLC analysis using 0.1% acetic acid : methanol (99 : 1) confirmed that biopterin was present in unoxidized head extracts [Fig. 2(A and B), peak 2]. Thus, spot no. 6 in Fig. 1 probably contained both natural biopterin from fly heads and biopterin formed by oxidation of 7,8-dihydrobiopterin.

Blue spot no. 7 (Fig. 1), a spot that was partially masked by a yellow spot (no. 12) on TLC plates, was identified as pterin. HPLC analysis using 0.1% acetic

acid : methanol (99 : 1) confirmed the presence of pterin in head extracts [peak 3 in Fig. 2(A and B)].

Blue spot no. 8 (Fig. 1) had low *R_f* values that corresponded to pterin 6-carboxylic acid. This compound is thought to be the final product of degradation of unstable C-6 pteridines as was reported by Ferre *et al.* (1986). HPLC analysis using 0.1% acetic acid : methanol (99 : 1) confirmed the presence of this compound in very small quantities in unoxidized samples.

Spot no. 9 (Fig. 1) had weak light blue fluorescence and did not correspond to any available pteridine standard either in colour of fluorescence or *R_f* value. Side by side comparison with synthetic DL kyurenine provided positive identification of this non-pteridine fluorescent compound as kyurenine. The presence of this chemical in extracts prepared for pteridine characterization was reported by Ferre *et al.* (1986) as a by-product of the biosynthetic pathway of xanthommatin.

Spot no. 10 (Fig. 1) had intense bluish green fluorescence and was not evident on all plates. We were unable to identify this compound, but there was some indication that it was a degradation product of sepiapterin. *R_f* values of this undetermined compound were similar to *R_f* values of a compound that sometimes separated from sepiapterin synthetic standards that were stored for a long period of time.

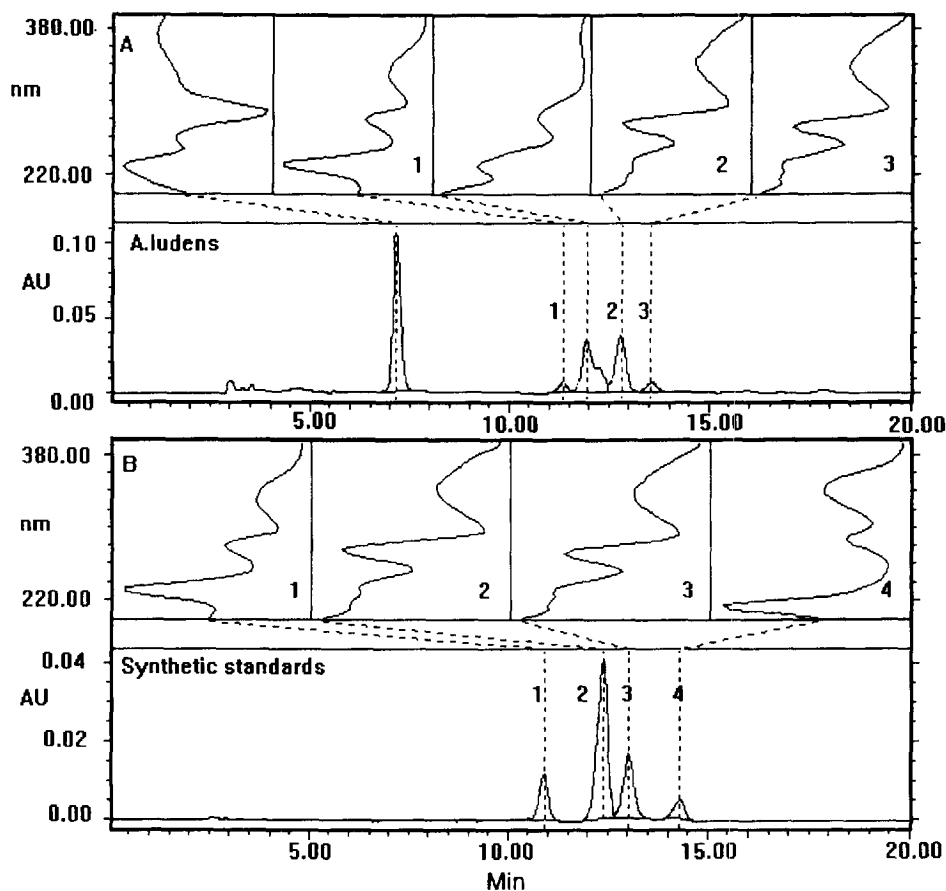


FIGURE 2. HPLC chromatograms at 360 nm and absorption spectra of compounds in head extracts of *A. ludens* (A) and of a synthetic standard mixture (B). The standard mixture contained 7,8-dihydrobiopterin (1), biopterin (2), pterin (3), and isoxanthopterin (4), each at 1 μ g/ml in pH 10 buffer.

Except for the drosopterins, the only pteridines found in the head capsule of *A. ludens* that had color under visible light were yellow pteridines. Two spots with yellow fluorescence were distinctive on the plates under UV light (Fig. 1). Spot no. 11 had the more intense fluorescence and had Rf values matching sepiapterin. Spot no. 12 had dull yellow fluorescence and Rf values corresponding to deoxysepiapterin. These two compounds were also readily separated using solvent system 2 where they again matched Rf values of sepiapterin and deoxysepiapterin. HPLC analysis using 30% methanol in water as the mobile phase confirmed the presence of these compounds (Fig. 3). This was demonstrated by comparison of retention times and absorption spectra of sepiapterin (peak 1) and deoxysepiapterin (peak 2) in Fig. 3(B) with retention times and absorption spectra of peaks 1 and 2 from fly-head extracts shown in Fig. 3(A).

Accumulation of pteridines with age

The experiment to determine the effect of fly age on titer of pteridines in fly heads showed that amounts of sepiapterin and deoxysepiapterin change with chronological age. Figure 4 shows the general trends of the accumulation of these two compounds when adult flies were maintained on 28°C. Each data point represents the titer of these compounds from a single fly head. These

data were fit using a power function regression as a theoretical model of chemical accumulation to an anticipated asymptote. These regressions were highly significant for estimating both sepiapterin ($F = 33.7$ at 1 and 108 d.f.; $P < 0.001$) and deoxysepiapterin ($F = 342.2$ at 1 and 108 d.f.; $P < 0.001$) given the age of the fly. However, the predictive value for sepiapterin was low due to excessive variability across all ages [Fig. 4(A)]. Predictive value for deoxysepiapterin was much better as indicated by the higher value of r^2 [Fig. 4(B)]. Our effort to explain this variability by taking into consideration the dry weight of fly heads and their sex was unsuccessful. Extending this method to estimate the age of flies based on levels of these compounds for practical application requires further study. Our current research efforts to do so involve determining the effects of different environmental factors such as temperature, adult diet, larval diet and photoperiod on the accumulation of both deoxysepiapterin and sepiapterin.

DISCUSSION

We were able to isolate 10 pteridines from the head capsule of Mexican fruit fly. All of the determined compounds have also been found in other examined dipteran species (Mail and Lehane, 1988). Interestingly, the pteri-

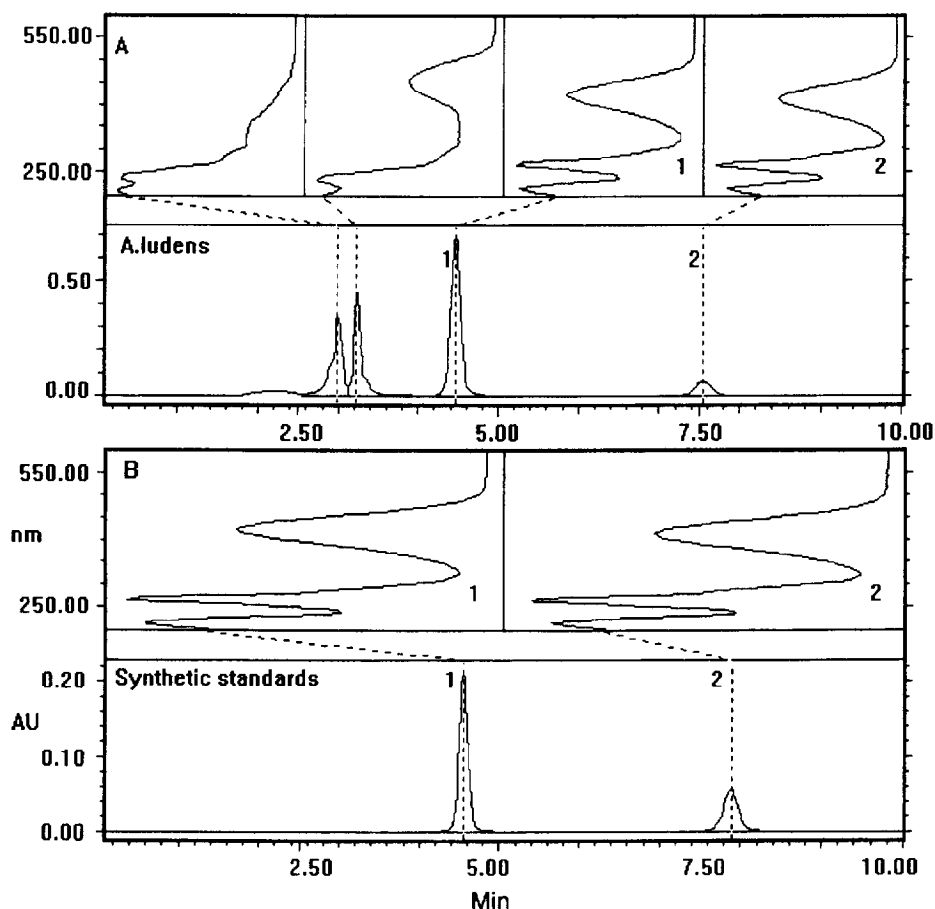


FIGURE 3. HPLC chromatograms at 420 nm and absorption spectra of compounds in head extracts of *A. ludens* (A) and of a synthetic standard mixture (B). The standard mixture contained sepiapterin (1) and deoxysepiapterin (2), each at 1 mg/ml in pH 10 buffer.

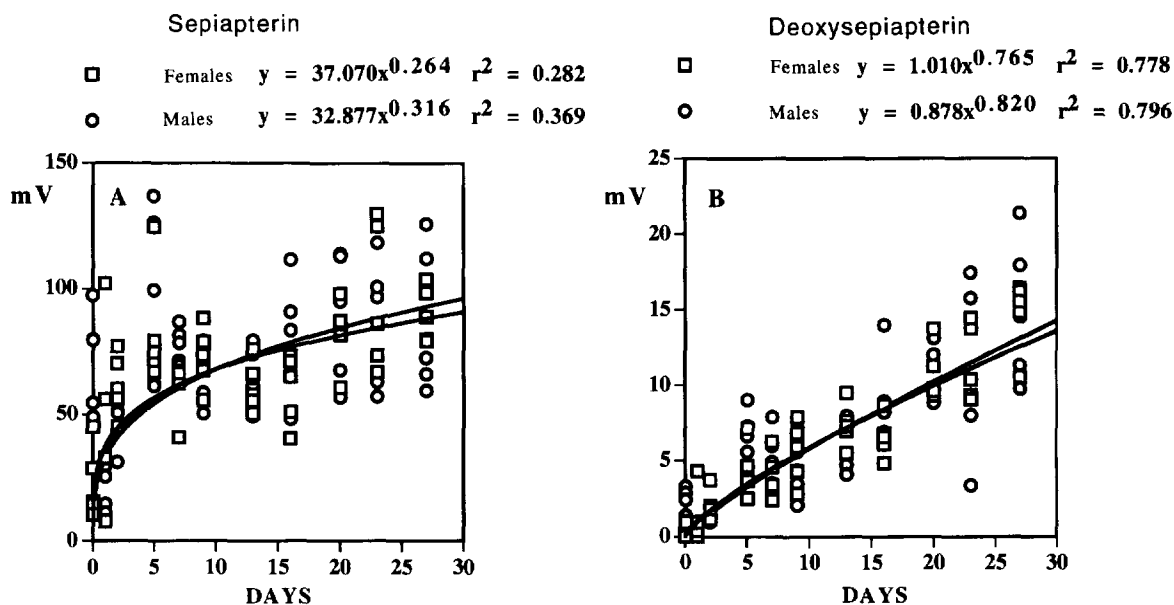


FIGURE 4. Accumulation with time of sepiapterin (A) and deoxysepiapterin (B) in head capsules of *A. ludens* reared at 28°C.

dine content from the head capsule of the Mexican fruit fly is similar to that of *D. melanogaster*, suggesting similar metabolism of pteridines in the Drosophilidae and Tephritidae.

Silva *et al.* (1991) proposed a biosynthetic pathway for pteridines in *Drosophila*. The pathway leads from guanosine triphosphate to H4-pyruvol pterin. The latter is a common precursor for several branches leading to four end products: 5,6,7,8-tetrahydrobiopterin; isoxanthopterin; drosopterins; and biopterin. Two of the four end products, drosopterins and biopterin, were detected in head extracts of *A. ludens*. 5,6,7,8-Tetrahydrobiopterin and isoxanthopterin were not observed.

Taking into consideration the inherent instability of 5,6,7,8-tetrahydrobiopterin and the techniques that were used, the prospect of tracing it in our study was low. The occurrence of pterin-6-carboxylic acid, a known oxidation product of 5,6,7,8-tetrahydrobiopterin (Ziegler and Harmsen, 1969), suggests that 5,6,7,8-tetrahydrobiopterin was probably present in fresh samples of *A. ludens* head extracts. No serious attempts were made to separate or detect this pteridine because the main goal of our study was to identify pteridines that accumulate with age and which might be suitable for practical application in age determination. 5,6,7,8-Tetrahydrobiopterin, a physiologically active and unstable pteridine required as a cofactor in enzymatic processes (Ziegler and Harmsen, 1969), did not appear to be a viable candidate for that purpose.

A second branch in the metabolic pathway proposed by Silva *et al.* (1991) leads to isoxanthopterin through its precursor pterin. Although isoxanthopterin was not detected in head capsule extracts from either sex, we did find pterin. Pterin is highly fluorescent, but is present in relatively small quantities. Visual examination of TLC plates revealed little if any change in concentration of this compound with age. This observation was not tested by HPLC analysis.

Another branch in the proposed pathway leads to drosopterins (Silva *et al.*, 1991), pigments that contribute to eye color. Although present in very small amounts, four different red and orange drosopterins were evident in *A. ludens* head extracts. We could not detect a significant age-related increase in drosopterin content on TLC plates. It is possible that our technique was not sensitive enough to detect these changes. However, it is known that concentrations of drosopterins change with age in *D. melanogaster*. For example, Parisi *et al.* (1976) demonstrated that drosopterins began to accumulate just before eclosion and continued to do so for 7 days. A similar pattern of accumulation was reported by Fan *et al.* (1976). In the latter study, drosopterins accumulated intensively during the first days of adult life, reached a maximum at 2–4 days, then remained constant with further aging.

The last of the four metabolic pathway branches proposed by Silva *et al.* (1991) is the biopterin branch. According to the proposed *D. melanogaster* pteridine biosynthesis pathway, the last metabolic branch includes biopterin, sepiapterin and 7,8-dihydrobiopterin. We detected all three of these pteridines in *A. ludens* (see Fig. 2). Biopterin and sepiapterin, both highly fluorescent compounds, and 7,8-dihydrobiopterin, which oxidizes to the highly fluorescent compounds biopterin and possibly neopterins, are mainly responsible for the overall fluorescence in the head extracts of *A. ludens*.

Fan *et al.* (1976) showed that sepiapterin, like drosopterins, accumulated during the first days of adult life, reached a maximum at 2–4 days, then remained constant with further aging in *D. melanogaster*. Our study also showed that sepiapterin accumulated with age in *A. ludens* (Fig. 4). The accumulation in the head capsule started just before eclosion (unpublished data) and reached a maximum at 7–10 days at 28°C. After that period, amounts of sepiapterin did not increase further.

Mail and Lehane (1988) attempted to improve the standard pteridine-fluorescence method of age determination in Diptera by separating and quantifying the highly fluorescent pterins extracted from flies of different ages. Our approach to improve the standard method for age determination differed from that of Mail and Lehane (1988). In *A. ludens*, the most highly fluorescent compounds changed little with age, as was discussed above. One dimensional TLC showed that deoxysepiapterin, one of the less fluorescent compounds found in *A. ludens* heads, changed markedly in titer with aging of the flies. TLC also provided good separation of deoxysepiapterin from other pteridines that masked the changes in concentration of deoxysepiapterin. However, TLC is labor intensive and quantification by TLC is not as accurate as was needed.

We chose HPLC for the separation because quantitation is both fast and accurate. Various mobile phases were tested for HPLC of deoxysepiapterin since no methods were available in the literature. The final mobile phase consisting of 30% methanol in water at a flow rate of 1 ml/min provided excellent separation such that retention time alone easily distinguished deoxysepiapterin from other compounds. In addition, the absorption spectrum of deoxysepiapterin was distinct from all other compounds except sepiapterin [peaks 1 and 2 in Fig. 3(B)] from which it is readily distinguished on the basis of retention time.

Although the structure of deoxysepiapterin was reported more than thirty years ago (Forrest and Nawa, 1962), little is known about this compound. No proposed metabolic pathway models include this pteridine. There is evidence that treatment of a mixture of sepiapterin and sepiapterin reductase with dilute acid produced biopterin and small amounts of deoxysepiapterin (Katoh and Akino, 1966). Also, it was reported that deoxysepiapterin can be made non-enzymatically from 7,8 dihydropterin and α -keto butyric acid in a reaction catalyzed by thiamine (Kaufman, 1962). Unfortunately these were only isolated attempts to explain the presence of this compound. No comprehensive investigations of deoxysepiapterin in Diptera have been reported.

This is the first study of deoxysepiapterin demonstrating a specific pattern of its function in an organism (i.e. that it accumulates with age in *A. ludens*). Further, its pattern of accumulation differed from that of sepiapterin in that the increase continued through at least 30 days of adult life. This characteristic makes deoxysepiapterin a promising candidate for practical use in age determination of captured specimens of *A. ludens* and possibly other fruit flies. In addition, our observations may stimulate new research concerning the place and function of deoxysepiapterin in the metabolism of pteridines.

REFERENCES

- Camin V., Baker P., Carey J., Valencuela J. and Arredondo-Peter R. (1991) Biochemical age determination for adult Mediterranean fruit flies (Diptera: Tephritidae). *J. Econ. Ent.* **84**, 1283–1288.
- Cheke R. A., Dutton M., Avissey H. S. K. and Lehane M. J. (1990) Increase with age and fly size of pteridine concentrations in different members of the *Simulium damnosum* species complex. *Acta Leind.* **59**, 307–314.
- Collins R. P. and Kalnins K. (1970) Pteridines in the moth, *Atteva punctella*. *J. Insect. Physiol.* **16**, 1587–1589.
- Fan C. L., Hall L. M., Skrinska A. J., and Brown G. M. (1976) Correlation of guanosine triphosphate cyclohydrolase activity and the synthesis of pterins in *Drosophila melanogaster*. *Biochem. Genet.* **14**, 271–280.
- Ferre J., Silva F., Real D. M. and Mensua J. L. (1979) Comparative study of the eye colour mutants of *Drosophila melanogaster*: quantification of the eye-pigment and related metabolites. In *Chemistry and Biology of Pteridines* (Eds Kisiliuk R. L. and Brown G. M.) pp. 669–673. Elsevier North Holland, Amsterdam.
- Ferre J., Silva F., Real M. D., Mensua J. L. (1986) Pigment patterns in mutants affecting the biosynthesis of pteridines and xanthomatin in *D. melanogaster*. *Biochem. Genet.* **24**, 545–569.
- Forrest H. S. and Nawa S. (1962) Structures of sepiapterin and isosepiapterin. *Nature* **196**, 372–373.
- Harmsen R. (1966) The excretory role of pteridines in insects. *J. Exp. Biol.* **45**, 1–13.
- Hearl W. G. and Jacobson K. B. (1984) Eye pigment granules of *Drosophila melanogaster*. *Insect. Biochem.* **14**, 329–335.
- Hopkins F. G. (1895) The pigments of Pieridae: a contribution to the study of excretory substances which function in ornament. *Phil. Trans. B.* **186**, 661–682.
- Katoh S. and Akino M. (1966) *In vitro* conversion of sepiapterin to isosepiapterin via dihydrobiopterin. *Experientia* **22**, 7.
- Kaufman S. (1962) On the structure of the phenylalanine hydroxylation cofactor. *J. Biol. Chem.* **237**, 2712–2713.
- Klein R., Tatischaff I., Tham G., Groliere C. A. (1991) The major pterin in *Tetrahymena pyriformis* is 6-(D-threo-1,2-dihydroxypropyl)-pterin(ciliapterin). *Biochimie* **73**, 1281–1285.
- Krafsur E. S., Rosales A. L., Robinson-Cox J. F. and Turner (1992) Age structure of horn fly (Diptera: Muscidae) populations estimated by pterin concentrations. *J. Med. Ent.* **29**, 678–686.
- Langley P. A., Hall M. J. R. and Felton T. (1988) Determining the age of tsetse flies *Glossina* spp. (Diptera: Glossinidae): an appraisal of the pteridine fluorescence technique. *Bull. Ent. Res.* **78**, 387–395.
- Lehane M. J. and Mail T. S. (1985) Determining the age of adult male and female *Glossina morsitans morsitans* using a new technique. *Ecol. Ent.* **10**, 219–224.
- Lehane M. J., Chadwick J., Howe M. A. and Mail T. S. (1986) Improvements in the pteridine method for determining age in adult male and female *Stomoxys calcitrans* (Diptera: Muscidae). *J. Econ. Ent.* **79**, 1714–1719.
- Mail T. S. and Lehane M. J. (1988) Characterization of pigments in the head capsule of the adult stablefly *Stomoxys calcitrans*. *Ent. Exp. Appl.* **46**, 125–131.
- Mail T. S., Chadwick J. and Lehane M. J. (1983) Determining age of adults of *Stomoxys calcitrans* (L.) (Diptera: Muscidae). *Bull. Ent. Res.* **73**, 501–525.
- Merlini L. and Nasini G. (1966) Insect pigments IV pteridines and colour in some Hemiptera. *J. Insect Physiol.* **12**, 123–127.
- Nixon C. J. (1985) Naturally occurring pterins. In *Folatet and Pterins* (Eds Blakely L. R. and Benkovic J. S.) pp. 2–41. John Wiley, New York.
- Parisi G., Carfagna M. and D'Amora D. (1976) A proposed biosynthesis pathway of drosopterins in *Drosophila melanogaster*. *J. Insect. Physiol.* **22**, 415–423.
- Rhode R. H. and Spishakoff L. M. (1965) Técnicas usadas en el cultivo de *Anastrepha ludens* (Loew). II memoria del día del Parasitólogo Chapingo Mexico.
- Silva J. F., Escriche B., Orondo E. and Ferre J. (1991) Genetic and biochemical characterization of little isoxanthopterin (lix), a gene controlling dihydropterin oxidase activity in *Drosophila melanogaster*. *Molec. Gen. Genet.* **230**, 97–103.
- Thomas D. B. and Chen A. C. (1989) Age determination in the adult

- screwworm (Diptera: Calliphoridae) by pteridine levels. *J. Econ. Ent.* **82**, 1140–1144.
- Wall R., Langley P. A., and Morgan K. L. (1991) Ovarian development and pteridine accumulation for age determination in the blowfly *Lucilia sericata*. *J. Insect Physiol.* **37**, 863–868.
- Wall R., Langley P. A., Stevens J. and Clarke G. M. (1990) Age determination in the old-world screw-worm fly *Chrysoma bezziana* by pteridine fluorescence. *J. Insect. Physiol.* **36**, 213–218.
- Wilson T. G. and Jacobson K. B. (1977) Isolation and characterization of pteridines from heads of *Drosophila melanogaster* by a modified thin-layer chromatography procedure. *Biochem. Genet.* **15**, 307–320.
- Ziegler I. and Harmsen R. (1969) The biology of pteridines in insects. *Adv. Insect Physiol.* **6**, 139–203.

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